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AQUATIC MICROBIOLOGY

Ultrastructural Organization of a Novel Halotolerant Strain *Kocuria* sp. ICIS A2.2 (Actinobacteria) after a Change of Carbon Source

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Abstract—Morphological and physiological properties, the partial sequence of the 16S rRNA gene, and the ultrastructure of a novel halotolerant hydrocarbon oxidizing bacterium *Kocuria* sp. (strain ICIS A2.2) isolated from the association with the ciliate *Cyclidium* sp. have been characterized. Morphological and ultrastructural changes have been revealed in cells of the studied strain while growing in a media with different carbon sources (sucrose or diesel fuel). At the cellular level, the enlargement of the cells, changes in their shape, and the formation of aggregates occur. At the subcellular level, both the number and size of cytoplasmic membrane bodies increase and electron-transparent inclusions appear. These changes may be considered a result of adaptation to a medium containing hydrocarbons.

Keywords: hydrocarbon oxidizing bacteria, *Kocuria* sp., cell ultrastructure, 16S rRNA gene

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INTRODUCTION

In recent years, microorganisms in soils and waters contaminated with oil and oil products have been actively studied. Oil-contaminated habitats are very diverse in microbial composition, and hydrocarbon-oxidizing microorganisms are found in different taxonomic groups [4, 8, 15]. The utilization of hydrocarbons as a carbon source by bacteria is associated with the changes in cellular metabolism, which in turn is accompanied by changes in cellular morphology and ultrastructure [2, 17]. The ultrastructure of genera *Rhodococcus*, *Arthrobacter*, and *Nocardia* is the most thoroughly studied among hydrocarbon-oxidizing bacteria [5, 19], while other hydrocarbon-oxidizing microorganisms are poorly studied. Representatives of the genus *Kocuria* are often found in hydrocarbon-oxidizing communities of aquatic ecosystems [11, 12]; however, data regarding the changes in ultrastructural organization of cells of these microorganisms grown on different carbon sources are absent in the literature.

The goal of this work was to evaluate changes in the ultrastructure of the cells of hydrocarbon oxidizing bacterium *Kocuria* sp. caused by a change in carbon source.

MATERIALS AND METHODS

The strain of hydrocarbon-oxidizing bacterium *Kocuria* sp. ICIS A2.2 Stackebrandt et al. 1995 isolated from a pure culture of free-living infusorium *Cyclidium* sp. O.F. Müller, 1773 was the subject of the study. The culture infusorium *Cyclidium* sp. was obtained from an ephemeral water reservoir with a salinity of 27.8 g/L located in the area of Tuzlukkol (Belyaevsky district, Orenburg oblast). To remove concomitant extracellularly located bacteria, the culture of *Cyclidium* sp. was treated with a mixture of antibiotics (100 μg/mL)—cefotaxime (Kraspharma, Russia) and gentamicin (Dalkhimpharm, Russia)—for 1 day. To remove antibiotics, the culture was washed three times by centrifugation (3000 rpm, 15 min, 4°C). The concentrated culture of the infusorium (100 μL) was inoculated by a spatula on Petri dishes with 1.5%-nutrient agar and Columbia agar (Fluka). The dishes were incubated for 24–48 h at 23–25°C. Three bacterial strains were isolated. The purity of the isolated cultures was checked by a visual assessment of the colonies microscopic examination of the smear, Gram staining, and the sequencing results (the absence of erroneous and unrecognized nucleotides). All strains were tested for their capability of utilizing hydrocarbons by means of cultivation in a Raimond liquid medium (NH_4Cl, 2 g;

† Deceased.